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An Unexpected Role for Ubiquitylation of a Transcriptional Activator

The yeast transcriptional activator Gal4 has served as a paradigm for understanding how eukaryotic cells mount rapid transcriptional responses to environmental changes. In this issue of *Cell*, Muratani et al. (2005) provide evidence that Gal4 ubiquitylation and destruction are required for activation by Gal4. Surprisingly, this modification is required at a postinitiation step in transcription for the production of mRNAs that are correctly processed and fully functional for translation.

Transcription by RNA polymerase II (pol II) involves a coordinated interplay among multiple classes of transcription factors, including activators and repressors, coactivators and corepressors, general transcription factors, and chromatin components. Changes in gene expression often occur through the posttranslational modification of these proteins. A modification that has received growing attention in this field is protein ubiquitylation (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). Whereas addition of polyubiquitin chains targets a protein for degradation by the proteasome, monoubiquitylation can alter a protein's function without signaling its destruction. Both forms of ubiquitylation have been observed among proteins involved in transcription. Similarly, the proteasome itself has been shown to regulate transcription initiation and elongation through both proteolytic and nonproteolytic mechanisms (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). Monoubiquitylation of histone H2B is associated with activation of transcription as well as transcription elongation (Xiao et al., 2005 and references therein). Polyubiquitylation of pol II occurs in response to DNA damage and is thought to signal the destruction of an irreversibly stalled elongation complex, enabling DNA repair and subsequent rounds of transcription (Muratani and Tansey, 2003). Finally, many transcriptional activator proteins are polyubiquitylated (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). For many of these factors, as expected, degradation reduces their function. However, for other activators, particularly those involved in growth control, ubiquitylation is *required* for transcriptional activation. There is a striking coincidence between the locations of transcriptional activation domains and the sites of ubiquitylation within these proteins, and activator strength is inversely related to activator abundance. However, while it has been speculated that the coupling of an activator's function to its destruction ensures tight control of transcription, the actual mechanism by which activator turnover controls transcription has not been elucidated.

In a comprehensive analysis, Muratani et al. (2005) address the roles of ubiquitylation and stability with respect to the function of the well-studied yeast activator Gal4. Decades of study have shown that Gal4 binds to specific sites in the regulatory regions of the *GAL* genes and activates transcription to a high level when *S. cerevisiae* is grown with galactose as a carbon source.

In their studies of the possible role of turnover in activation by Gal4, Muratani et al. (2005) first elucidate a way to study "active" Gal4, the subset of Gal4 bound to its sites under activating conditions. Their analysis, in agreement with past studies, identified three Gal4 isoforms, Gal4a, Gal4b, and Gal4c, created by differential phosphorylation (Hirst et al., 1999; Mylin et al., 1990; Sadowski et al., 1991). Several experiments suggest that Gal4c, present only in galactose-grown cells, is the active form. The phosphorylation events that create Gal4c are a consequence of activation; thus, while phosphorylation is not required for Gal4 activity, it allows the study of active Gal4 with respect to ubiquitylation.

Analysis of the stability of each Gal4 isoform in both raffinose (a noninducing carbon source) and galactose revealed that Gal4 stability is differentially controlled by carbon source. In raffinose, the two Gal4 isoforms present, Gal4a and Gal4b, are unstable with a half-life of approximately 20 min. This instability is dependent upon Grr1, an F box ubiquitin ligase, as a *grr1Δ* mutation causes stabilization of Gal4a and Gal4b. The *grr1Δ* mutation also causes activation of *GAL1* under these noninducing conditions, likely due to increased Gal4 levels. In galactose, different results are seen—Gal4a and Gal4b are stable, and Gal4c is highly unstable, with a half-life of less than 5 min. In galactose, Gal4c instability is independent of Grr1, but it is dependent upon a different F box protein, which the authors call Dsg1. Dsg1 was previously identified as Mdm30, a factor required during mitochondrial fusion (Fritz et al., 2003). In a *dsg1/mdm30* null mutant, Gal4c is highly stable. Furthermore, Gal4 polyubiquitylation, detected in wild-type cells, is absent in the *dsg1/mdm30Δ* mutant. Interestingly, the mutant is also Gal^-, indicating a role for Dsg1/Mdm30 and, presumably, Gal4 turnover in Gal4 activation.

Two sets of experiments strongly point toward a direct role for Dsg1/Mdm30 in Gal4 activation. First, in a *dsg1/mdm30Δ* mutant, activation of a *GAL1-lacZ* fusion is defective, based on β-galactosidase and Western assays. This defect is specific for the Gal4 transcriptional activation domain, as the Myc transcriptional activation domain, when fused to the Gal4 DNA binding domain, functions normally in a *dsg1/mdm30Δ* mutant. Second, chromatin immunoprecipitation experiments show that Dsg1/Mdm30 is physically associated with the *GAL1* regulatory region under inducing conditions.

Unexpectedly, additional experiments by Muratani et al. (2005) suggest that Dsg1/Mdm30 controls *GAL1* expression at a postinitiation step in transcription; when compared to wild-type cells, the *dsg1/mdm30Δ* mutant does not have reduced levels of *GAL1* mRNA. However, several other defects do occur in the *dsg1/mdm30Δ*

mutant. Most prominently, the level of phosphorylation of the pol II C-terminal domain (CTD) is significantly decreased both at Ser2 and Ser5, the positions in the CTD repeat usually phosphorylated in elongating pol II (Sims et al., 2004). As CTD phosphorylation is required for the recruitment of several factors required for mRNA maturation, this defect is likely the cause of the other defects detected, including the lack of association of *GAL1* mRNA with polysomes. Taken together, these results suggest that Gal4 ubiquitylation and turnover play a key role in Gal4 activation at a level that affects mRNA maturation. The authors suggest a model in which Gal4 destruction promotes disassembly of the initiation complex, facilitating a transition to a productive elongation complex.

A number of important issues are illuminated by this work and suggest future experiments. A central question that remains to be addressed is whether Gal4c is the only target of Dsg1/Mdm30 that is relevant to *GAL* gene activation. While Muratani et al. (2005) clearly demonstrate effects of Dsg1/Mdm30 on Gal4 ubiquitylation and stability, it remains possible that Dsg1/Mdm30 also modifies and destabilizes a more globally acting transcription, chromatin, or RNA processing factor. A challenging experiment, to map and mutate the Dsg1/Mdm30-dependent Gal4 ubiquitylation sites, would address whether Gal4 ubiquitylation is the whole story with respect to the effects observed.

Assuming that Gal4 is the relevant target, it will be important to determine whether ubiquitylation per se or ubiquitin-dependent proteolysis is required for activation. In an earlier study, direct fusion of ubiquitin to Gal4-VP16 compensated for the loss of normal ubiquitylation of this activator (Salghetti et al., 2001). The resulting fusion protein was competent for activation yet stable, demonstrating that the contributions of protein ubiquitylation and degradation can be uncoupled. Modification of the Gal4 transcription activation domain by Dsg1/Mdm30-mediated ubiquitylation could potentially affect the recruitment of specific factors required for subsequent events. An interesting possibility is that Dsg1/Mdm30 may recruit components of the proteasome, which have been argued to play nonproteolytic roles in transcription elongation (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). If Gal4 turnover is indeed essential for activation, this would suggest that activated Gal4 must be regenerated with each new round of transcription. For the *GAL* genes, then, each round of transcription could be viewed as a "pioneer" round requiring reassembly of an initiation complex.

Given the importance of Gal4 ubiquitylation, the regulation of Dsg1/Mdm30 itself becomes an important issue. Is association of Dsg1/Mdm30 with the *GAL1-GAL10* UAS regulated by galactose or dependent upon known coactivator complexes, such as SAGA? Is Dsg1/Mdm30 itself part of a known coactivator complex? Does it activate Gal4 in conditions where galactose is not needed for *GAL* gene induction, such as in a *grr1Δ* mutant grown in raffinose?

Finally, and most importantly, the mechanism by which activator function is tied to a productive transition from initiation to elongation remains to be elucidated. Future studies will certainly focus on CTD phosphorylation, whose levels are significantly reduced in strains lacking Dsg1/Mdm30 and whose central role in coordinating RNA synthesis and maturation is well established. An investigation of how the ubiquitylation and elimination of a most well-studied activator, Gal4, leads to proper CTD phosphorylation and progression of the transcription cycle will likely provide general insights into the control of gene expression. The work of Muratani et al. (2005), therefore, both adds significantly to our knowledge in the area of transcriptional regulation and sets the stage for what will likely include additional surprising results.

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